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Original paper

Diagnosis of bovine gastrointestinal parasites: comparison of different techniques and different solutions

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ABSTRACT. Cattle are important livestock species for protein and income supply. But disease agents, including gastrointestinal (GI) parasites, hinder their productivity. For effective GI parasites control, there is need for rapid, highly sensitive and specific diagnostic techniques and tools, lack of which poses problem to clinician, diagnostic staff and livestock owners. One hundred cattle faeces were analyzed through Simple Faecal Flotation (SFF), Modified Centrifugal Faecal Flotation (MCFF) and Mini-FLOTAC techniques (MFT) using sodium chloride, brine, sugar, salt/sugar and zinc sulphate at specific gravity of 1.2, 1.23, 1.25, 1.3 and 1.3 respectively as Flotation Solutions (FS). Overall GI parasite prevalence was 100%. Parasite elements identified were: Strongyle eggs (99%), *Strongyloides papilosus* (97%), *Neoascaris vitulorum* (78%), *Trichostrongylus* (56%), *Nematodirus* (46%), *Capillaria* spp. (14%) and *Trichuris* spp. (6%), *Moniezia benedeni* (24%), *Moniezia expansa* (16%), *Taenia*-like eggs (3%), *Schistosoma* eggs (3%) and *Eimeria* oocysts (100%). Sensitivity of 61.99%, 58.49% and 54.24%. for MFT, SFF and MCFF respectively was gotten using Salt/Sugar. With these techniques, Salt/Sugar availability with affordability; its use as a routine FS in diagnosis of GI parasite is advocated. The ease and rapid of use of Mini-FLOTAC diagnostic Kit can be adopted in low economic countries like Nigeria where power supply is limited as well as the safety of user and possibility of re-usage.

Keywords: copro-analysis, cattle, Mini-FLOTAC, salt/sugar

Introduction

Gastrointestinal helminths are ubiquitous parasitic agents of livestock especially ruminants and are known to limit cattle production in many climatic areas and countries [1]. While the majority of helminth parasites reside in the intestines, they can also be seen in the stomach, bile duct, lungs, liver and even gall bladder of ruminants [2].

Helminth infection of ruminants are mostly caused by nematodes (*Haemonchus* spp., *Ostertagia* spp., *Trichostronglus* spp., *Cooperia* spp., *Trichuris* spp., *Capillaria* spp., *Strongyloides* spp.), trematodes (*Fasciola hepatica*, *Fasciola gigantica*, *Dicrocoelium* spp., *Paramphistomum* spp.) and cestodes (*Moniezia benedeni*, *Moniezia expansa*, *Avitellina* spp., *Thysaniezia* spp., *Taenia* spp.) [3].

There appears to be almost endless possibilities in the number of substances that could be used in the flotation technique. Indeed, everything from simple table salt and table sugar (sucrose) to other chemicals such as zinc sulphate (Faust's liquid), sodium nitrate, and combination solutions such as salt/sugar, mercury iodide/potassium iodide (Janeckso-urbanyl solution), and sucrose/sodium nitrate, have been used in flotation procedures. Although solutions with specific gravities of 1.15 to 1.55 have been investigated, efforts have generally been focused on solutions with specific gravities between 1.18 and 1.30 [4–7].

As polyparasitism is very common and in response to the recent trend toward integrated control of multiple parasitic diseases, there is a need for sensitive and accurate diagnostic tools that are simple to apply, as a result of this, Cringoli et al. [8] developed multivalent techniques, denominated FLOTAC, for qualitative and quantitative copro-microscopic diagnosis of parasitic infections in

animals and humans. The FLOTAC apparatus consists of a cylindrical device with 5ml floatation chamber, which allows up to one gram of stool to be prepared for microscopic analysis, that is, allows the quantification of as low as one egg per gram of faeces. The FLOTAC technique proved to be more sensitive compared to the McMaster technique in a survey of anthelminthic resistance in cattle [9] However, FLOTAC is more time consuming and requires a centrifuge for plates which limits its adoption for use where a centrifuge is not available or electric power supply is irregular, as is the case with several developing countries like Nigeria. To overcome this constraint, a more user-friendly technique, Mini-FLOTAC, was developed by the same researchers in 2013 [10]. Mini-FLOTAC does not require centrifugation and can detect as low as 10 eggs per gram of faeces. It produces highly reproducible results, and is particularly useful for monitoring and surveillance, for which large numbers of faecal samples must be rapidly, yet reliably, examined [10].

Fill-FLOTAC was designed along with mini-FLOTAC, the Fill-FLOTAC are sampling devices, which perform the first four consecutive steps of the Mini-FLOTAC technique, i.e. collection (including weighing) homogenization, filtration and filling. This technique permits analysis of both fixed and fresh faecal samples which allows the possibility of examining the samples on different days and improves the quality control process [8,11]. We are not aware of studies regarding the evaluation of this technique in Nigeria. Hence this study on the feasibility of employing the technique for the detection and quantitation of GIT parasite eggs in large ruminant faeces in the area of study.

Materials and Methods

Study sites, study population and selection of samples

One hundred faecal samples were collected from the rectum of cattle in Ipokia Local Government Area (LGA) and Odeda LGA of Ogun State, Nigeria. This number was calculated assuming a prevalence of 50% with a 95% confidence level since the prevalence of helminthosis is known to be high in the study area. Each sample was collected into a clean, labeled Universal sample bottle. All the samples were transported in an ice box to the Parasitology Laboratory College of Veterinary Medicine, Federal University of Agriculture, Abeokuta, for analysis**.**

Preparation of floatation solutions

Brine

Sufficient quantity of sodium chloride was added to one liter of distilled water in a container, the mixture was stirred until the salt could no longer dissolve. Presence of salt crystals (undissolved salt particles) after 24 hours was taken to mean that the solution could no longer dissolve the salt. An hydrometer was used to check the specific gravity which was adjusted to 1.23 [12].

Saturated sugar solution

454 g of granulated sugar was added to 355 ml of water in a container, the mixture was stirred until it was saturate as indicated by the presence of sugar crystals at the bottom of the container after continuous stirring for 15 minutes. Hydrometer was placed into the solution to check the specific gravity, this was adjusted to 1.25 before usage. Few drops of 40% formalin were added to the solution to serve as preservative and to keep ants away [13].

Salt/sugar solution

400g of sodium chloride was measured into a container with a liter of distilled water added. The mixture was continuously stirred until it dissolved. Then 500 g of sugar was added to the salt solution; and stirred until the sugar dissolved. Hydrometer was placed into the salt/sugar solution and the solution adjusted to a specific gravity of 1.30 [13].

Zinc sulphate solution

371g of zinc sulphate was measured into a container, 1000 ml of water was added, and then stirred. Hydrometer was used to adjust the solution to a specific gravity of 1.30 [13].

Sodium chloride solution

This was prepared by adding 400 g of sodium chloride to 1 liter of water and stirred until the salt dissolved in water. Hydrometer was used to adjust the specific gravity to 1.2 [13].

Fourteen different test analysis were performed on each faecal sample using three faecal examination techniques: simple Faecal Flotation (SFF), Modified Centrifugal Faecal Flotation (MCFF) and Mini-FLOTAC (MFT). Five different flotation solutions were used for MCFF and MFT techniques while four out of the five were used for SFF. The flotation solutions were: sodium chloride

(NaCl; SG 1.20), brine (SG 1.23), sugar (SG 1.25), salt/sugar (SG1.30) and zinc sulphate (ZnSO4; SG 1.30).

Parasitological techniques

SFF. One gram of faeces was placed into a mortar. The faecal flotation solution was added and the mixture was emulsified with pestle, the resulting mixture was poured through a strainer to remove faecal debris. The resulting faecal suspension was used to fill a 15 ml test tube to two-third capacity. The test tube was placed into a test tube rack and the tube was filled to the brim (until a convex meniscus was formed) with any of the FS to be used solution and a 22 mm×22 mm coverslip was placed over the convex meniscus. Twenty minutes was allowed for parasite elements flotation after which the coverslip was removed and placed on a glass slide for light microscopic examination at ×100 and x400 magnification [14].

MCFF. One gram of faeces was placed in a mortar, water was added, and the mixture emulsified using pestle. The faecal suspension was strained through a strainer into a beaker and the strained suspension was used to fill a 15 ml centrifuge tube with cover to two-third capacity. The test tube was centrifuge for 10 minutes at 250 g after which the supernatant was removed, leaving faecal sediment at the bottom of the tube. Specified flotation fluid was then added to the faecal sediment which was re-suspended and centrifuged for a further 5 minutes. The tube was then removed, placed into a test tube rack, filled to the rim (until a convex meniscus was formed) with the flotation solution. A 22mm×22 mm coverslip was placed over the formed meniscus and was allowed to stay 10 minutes for parasite elements flotation. The resultant coverslip was put on a slide and was examined under light microscope using ×100 and ×400 magnification [14,15].

Mini-FLOTAC. Five grams of fresh faeces were put into the Fill-FLOTAC container measured with a calibrated cone found in the kit and 45 ml of floatation fluid was added. The suspension was then thoroughly homogenized using the stick of the Fill-FLOTAC. The faecal suspension was then filtered through the Fill-FLOTAC and used to fill the two chambers of the Mini-FLOTAC reading disk. The reading disk was allowed to stand for 10 minutes for parasite elements to float. After 10 minutes, the top parts of flotation chambers were translated and the Mini-FLOTAC was read under a light microscope using 100× and 400× magnification [10].

Data analysis

Accruing data were subjected to descriptive statistical analysis using percentages to describe the prevalence rates across different breeds, age, body condition score and sex.

Results from this study were also applied by means of comparisons of positive data from faecal samples obtained by parasitological techniques, to determine, their sensitivity. Total positive from all the three techniques and the flotation solutions was taken to be the gold standard [16] to cater for the merits and demerits of each technique and floatation solutions.

Results

Table 1. The distribution of different bovine gastrointestinal parasites eggs and oocysts using three diagnostic techniques

Parasites	No. examined	No. positive (%)
Strongyle	100	99
Strongyloides	100	97
Trichostrongylus	100	56
Nematodirus	100	46
N. vitulorum	100	78
Trichuris	100	6
Capillaria	100	14
Moniezia benedeni	100	24
M. expansa	100	16
Taenia	100	3
Schistosoma	100	3
Eimeria oocyst	100	100

Out of the one hundred cattle sampled all were positive for at least one gastrointestinal parasite as helminth egg or protozoan oocysts (100%). Eleven genera of gastrointestinal parasites were recovered in this study. They include: seven nematodes – Strongyle (99%), *Trichostrongylus* (56%), *Nematodirus* (46%), *Neoascaris vitulorum* (78%), *Strongyloides* spp. (97%), *Capillaria* spp. (14%) and *Trichuris* spp. (6%)); two cestodes: *Moniezia benedeni* (24%), *Moniezia expansa* (16%) and *Taenia* eggs (3%); one trematode: *Schistosoma* spp. (3%) and one protozoa: *Eimeria* (100%) (Tab. 1).The distribution of all identified parasitic helminth eggs, shows that Strongyle eggs were the most prevalence (99%) of the nematode eggs recovered and *Trichuris* spp. was the least prevalent (6%). The only protozoa oocyst recovered was *Eimeria*, the prevalence of which was 100% (Tab.

Table 2. Distribution of bovine GIT parasites in relation to age, sex, breed and Body Condition Score (BCS)

Parameters	Age			Sex		Breed					BCS		
	Calf	Yearling	Adult	Male	Female	WF	MU	CR	SG	ND	L	M	G
No. of animal examined	21	38	41	59	41	53	29	04	01	13	19	73	8
No. positive	21	38	41	59	41	53	29	04	01	13	19	73	8

Explanations: Breed – WF: White Fulani, MU: Muturu, CR: Cross, SG: Sokoto Gudali, ND: N'dama; BCS – L: Lean, M: Moderate, G: Good

1). The distribution of gastrointestinal parasites in relation to age, breed, body condition and sex was 100% (Tab. 2).

Of the 100 faecal samples collected, 59 were males, and 41 were females. Based on the age group, 21 were calves, 38 were yearlings and 41 were adults. Using the body score, 19 of the sampled population were lean, 73 were moderate and only 8 were in good body condition. The breed of the sampled cattle were: White Fulani (53), Muturu (29), N'dama (13), Cross breed (4) and Sokoto Gudali (1) (Tab. 2).

The following prevalence were observed through the use of three parasitological techniques and five flotation solutions; Simple Faecal Flotation using salt, sugar and salt/sugar (salt SFF, sugar SFF and salt/sugar SFF), Modified Centrifugal Faecal Flotation using sugar and salt/sugar (sugar MCFF and salt/sugar MCFF) and Mini-FLOTAC Technique using salt, sugar and salt/sugar (salt MFT, sugar MFT and salt/sugar MFT) presented the same diagnostic positivity of 100% (Tab. 3).

Table 3. Prevalence and number of parasitic infections found in the faeces

Diagnostic methods		Single	Double	Triple	Quadruple	Quintuple	Sextuple	Total	% Positive
SFF	Salt SF	20	31	33	13	3	0	248	100
	Brine SF	22	39	23	7	3	0	211	94
	Sugar SF	10	31	43	13	2	1	271	100
	Salt/sugar SF	1	24	46	18	10	1	316	100
MCFF	Salt MCFF	31	28	14	12	4	0	198	89
	Brine MCFF	29	23	18	16	3	0	208	89
	Sugar MCFF	16	31	33	13	7	0	264	100
	Salt/sugar MCFF	14	24	26	27	9	0	294	100
	$ZnS0_4$ MCFF	12	33	32	12	2	0	232	91
MFT	Salt MFT	24	37	19	12	6	2	247	100
	Brine MFT	14	38	27	14	5	1	258	99
	Sugar MFT	11	30	29	15	12	3	297	100
	Salt/sugar MFT	3	17	33	33	12	2	336	100
	$ZnSO_4$ MFT	7	23	43	23	2	0	286	98

Explanations: SFF: Simple Faecal Flotation, MCFF: Modified Centrifugal Faecal Flotation, MFT: Mini-FLOTAC technique

While salt MCFF and brine MCFF (S.G 1.2 and 1.23, respectively), gave 89% prevalence, Mini-FLOTAC using brine and zinc sulphate, gave 99% and 98% respectively. Simple Faecal Flotation using brine (Brine SFF) and Modified Centrifugal Faecal Flotation using zinc sulphate (zinc sulphate MCFF) had 94% and 91%, respectively. However, the number of infections detected, was higher with Mini-FLOTAC using salt, sugar and salt/sugar as flotation solutions. The Mini-FLOTAC detected

Table 4. Parasites detected versus Gold Standard (GS*)

	Simple Faecal Flotation				Modified Centrifugal Faecal Flotation					Mini-FLOTAC Technique					
Parasites	Salt	Brine	Sugar	Salt /sugar	Salt	Brine	Sugar	Salt/ sugar	Znso4	Salt	Brine	Sugar	Salt/ sugar	$ZnS0_4$	GS*
Strongyle	90	86	94	96	78	79	84	73	76	91	86	88	79	82	99
Strongyloides	45	36	62	88	32	41	55	74	70	24	30	41	74	85	97
Trichostrongylus	22	19	05	15	13	20	05	05	02	17	19	10	03	03	56
Nematodirus	22	13	03	07	04	03	03	02	01	20	16	03	07	06	46
N. vitulorum	0	04	07	11	02	0	26	34	15	05	06	44	53	09	78
Capillaria	0	0	0	02	03	0	01	04	01	03	01	06	03	02	14
Trichuris	0	0	03	0	0	0	02	0	01	0	0	01	01	0	06
Moniezia benedeni	09	08	11	12	08	11	08	09	09	10	15	10	11	09	24
M. expansa	02	01	02	03	01	0	01	0	0	02	03	02	06	05	16
Taenia	0	0	0	0	0	0	0	0	0	0	0	01	02	0	03
Schistosoma	0	0	0	0	0	0	0	0	0	0	0	0	02	01	03
Eimeria	58	44	84	83	57	54	79	93	57	75	82	91	95	84	100
Total	248	211	271	317	198	208	264	294	232	247	258	297	336	286	542

single, double, triple, quadruple, quintuple or sextuple infection in the study samples (Tab. 4).

Eleven genera of parasites, ten of helminths and one of protozoa, were identified by fourteen faecal analytical techniques making a total of 1400 clinical analyses. Only the Mini-FLOTAC technique with salt/sugar flotation solution (salt/sugar MFT) was able to detect all the eleven genera. It is therefore comparable to the Gold Standard, since it showed the highest accuracy in the identification of 336 positive analyses.

In accordance with the Gold Standard, among the group of helminths, *Neoascaris vitulorum* predominated, being detected in 78 (78%) of the cattle. In the detection of different parasite genera, Mini-FLOTAC using salt/sugar excelled over the other techniques, revealing infection in 53 (53%) cattle compared to the 34 (34%) cattle detected by the Modified Centrifugal Faecal Flotation using salt/sugar (salt/sugar MCFF), 11(11%) cattle by the Simple Flotation Flotation using salt/sugar (salt/sugar SF). The rate of detection by other techniques were: Mini-FLOTAC using sugar (sugar MFT), 44%, Modified Centrifugal Faecal Flotation using sugar (sugar MCFF), 26%, Simple Faecal Flotation using salt/sugar (salt/sugar SFF) 11%, and Mini-FLOTAC using zinc sulphate (zinc sulphate MFT) 9% (Tab. 4).

In the detection of Strongyle and *Strongyloides* spp., the salt/sugar Simple Faecal Flotation method gave the best performance of 96% and 88% respectively. Salt/sugar Mini-FLOTAC method gave the best performance in detecting *Taenia* eggs, *Schistosoma* spp. and *Eimeria* oocysts of 2%, 2% and 95% respectively compared to other methods. The highest number of *Capillaria* spp. was detected by sugar Mini-FLOTAC method (6%) (Tab. 4).

In terms of the diagnostic performance of the techniques, using sensitivity, the salt/sugar Mini-FLOTAC showed sensitivity of 61.99% compared to 58.49%, 57.80%, 54.24%, 52.77%, 50.0%, 48.71%, 47.60%, 45.76%, 45.57%, 42.80%, 38.93%, 38.38% and 36.53% of salt/sugar SFF, sugar MFT, salt/sugar MCFF, Znso4 MFT, sugar

Table 5. Diagnostic performance (sensivity %) of the three parasitological techniques

Techniques	Simple Faecal Flotation				Modified Centrifugal Faecal Flotation					Mini-FLOTAC				
Solution	Salt	Brine	Sugar	Salt/ sugar	Salt	Brine	Sugar	Salt/ sugar	$ZnSO_4$	Salt	Brine	Sugar	Salt/ sugar	$ZnSO_4$
Sensitivity	45.76	38.93	50	58.49	36.53	38.38	48.71	54.24	42.8	45.57	47.60	54.80	61.99	2.77

SFF, sugar MCFF, brine MFT, salt SFF, salt MFT, Znso4 MCFF, brine SFF, brine MCFF and salt MCFF, respectively (Tab. 5).

In terms of rapidity of the three techniques, simple flotation takes two minutes for processing a sample, twenty minutes required for floating and one minute for observation under the microscope (23 minutes all together). Modified Centrifugal Faecal Flotation takes seventeen minutes for processing a sample, minimum of ten minutes for floating of parasite elements and one minute for microscopic examination (28 minutes all together). Mini-FLOTAC technique takes three minutes to process a sample, ten minutes for parasite elements to float and one minute for microscopic examination of the reading disk (14 minutes all together).

Discussion

The findings of this study showed that 100% (100/100) of the cattle screened had helminth infection, thus providing valuable information on the burden of helminths among cattle in sampled areas. Strongyle (nematode) infections were particularly high as it accounted for 99% of the total helminth burden. This high prevalence may be as a result of the number of techniques used and the time of the year in which the sample was collected (rainy season, April-September) for this study.

The overall prevalence of 100% of helminth infection obtained in this study was higher than those by Edosomwan and Shoyemi [17] and Adedipe et al. [18] who reported a prevalence of 47.4% and 41.6% in South-South and South-West Nigeria, respectively. It was also higher than 50.8% and 62.1% earlier reported in South-Eastern and South-South Nigeria by Elele et al. [2] and Nwigwe et al. [19], respectively. The differences observed could be due to the rainy period in which this study was conducted (April–September 2017), the sources of cattle sampled in the various areas, variation in sample size, since one hundred cattle were sampled compared to 129 and 397 animals sampled by Elele et al. [2] and Adedipe et al. [18] and the management system.

Furthermore, this study revealed that both the male and female animals have equal chance of being infected with gastrointestinal parasites, since the results obtained from this study showed that all the male and female animals sampled were infected. One of the major factors that would have accounted for this is the fact that both male and female cattle under local setting in Nigeria are exposed to poor feeding and veterinary care, factors accountable for equal susceptibility to helminth infections. This agrees with the findings of Adedipe et al. [18] that both male and female cattle have equal chance of being infected with gastrointestinal parasites.

The breed prevalence of 53.0%, 29%, 13%, 4% and 1% obtained for White Fulani (Bunaji) Muturu, N'dama, Crossbreed and Sokoto Gudali cattle respectively, were lower than the 62% (Bunaji) and 62.2% (Sokoto Gudali) as earlier reported by Elele et al. [2] and higher than 46.0% (Bunaji) reported by Adedipe et al. [18]. The difference in the prevalence obtained could be attributed to the existence of favourable environmental factors necessary for the prolonged survival and development of infective larval stage of most helminths Ekong et al. [20] variation in sample size and management system. Regassa et al. [21] reported that animals that are solely graze on pasture throughout the year are prone to the effect of seasonal variation of availability of forgeable feed and then difference in plane of nutrition.

The body condition score had no significant effect on prevalence of helminthosis in this study, as cattle with moderate, lean (poor) and good body condition score had equal prevalence (100%). This finding is different from that of Adedipe et al. [18] who reported that cattle with moderate body condition score had higher prevalence of gastrointestinal parasites when compared to those that were emaciated. The reasons given was that those with moderate body score tolerated helminth infections better or that both host and parasites had reached a state of equilibrium and were asymptomatic at the time of faecal collection [22]. Also, the sensitivity of method and the number of methods used may be responsible for the variation in prevalence recorded by different workers.

The parasite elements identified in this study were similar to those identified by Adedipe et al. [18], Edosomwan and Shoyemi [17] and Elele et al. [2] in earlier studies carried out in Ibadan, Benin and Port Harcourt, Nigeria respectively. Findings from these studies showed that 8, 12 and 16 different helminthes were obtained from Ibadan, Benin and Port Harcourt respectively and some of the helminthes are similar to those found in this study. The parasite elements common to both studies are Strongyle, *Strongyloides* spp., *Trichostrongylus*, *Trichuris* spp., *Moniezia* spp., *Nematodirus*, *Neoascaris vitulorum*, *Taenia* and

Schistosoma spp. This shows that these parasite elements are distributed everywhere irrespective of the geographical location of sampled animals, throughout the tropics and sub-tropical regions.

The parasite elements identified using Formol-ether concentration technique, by Elele et al. [2] are *Haemonchus* species, *Strongyloides* spp., *Chabertia*, *Trichuris* spp., *Ostertagia* spp., *Bunostomum* spp., *Trichostrongylus* spp., *Ascaris* spp., *Taenia*, *Moniezia* spp., *Avitellina* spp., *Dicrocoelium* spp., *Fasciola* spp., *Eurytrema* spp., *Gastrotylax* spp., and *Schistosoma* spp. compared to the Strongyle eggs, *Trichostrongylus*, *Nematodirus*, *Neoascaris vitulorum*, *Strongyloides* spp., *Capillaria* spp. and *Trichuris* spp., *Moniezia benedeni*, *Moniezia expansa*, *Taenia* eggs, *Schistosoma* eggs and *Eimeria* oocysts identified in this study using simple flotation, modified Centrifugal Faecal Flotation and Mini-FLOTAC techniques. Also using simple flotation and sedimentation techniques Adedipe et al. [18] detected the following parasite elements: Strongyle, *Strongyloides*, *Nematodirus*, *Neoascaris vitulorum*, *Moniezia* spp., *Dicrocoelium*, *Paraphistomum* spp. and *Fasciola* eggs compared to the Strongyle eggs, *Trichostrongylus*, *Nematodirus*, *Neoascaris vitulorum*, *Strongyloides* spp., *Capillaria* spp. and *Trichuris* spp., *Moniezia benedeni*, *Moniezia expansa*, *Taenia* eggs, *Schistosoma* eggs and *Eimeria* oocysts identified in this study using Simple Flotation, Modified Centrifugal Faecal Flotation and Mini-FLOTAC techniques.

This study showed that infections with nematodes were the most common and especially Strongyles were the most frequent compared with other types of worms. This finding was in agreement with what Adedipe et al. [18] reported, which stated that Strongyle-type eggs are the most prevalent among the nematode eggs identified. Moreover, the sampling was done during raining season when Strongyle eggs are known to be numerous in cattle herds.

An important observation from this study is that the *Capillaria* eggs detected is from Muturu breed of cattle sampled in rural Ipokia Local government Area. In this area, many people defecate on the farmland and these animals are grazed on the same farmland that had been contaminated with human faeces. For this reason, the *Capillaria* spp. detected from these animals may be of human origin with attendant zoonotic implication calling for further research in the study area. Although bovine coprological examination carried out by various researchers in other countries reported *Capillaria* spp. as one of the parasite elements detected [23], to date no such report has been made from cattle in Nigeria.

This study confirms that salt/sugar solution performs better under Modified Centrifugal Faecal Flotation Technique in the detection of *Eimeria* oocysts, *Neoascaris vitulorum* and *Capillaria* compared to Simple Faecal Flotation.

In the diagnosis of *Trichuris* eggs, sugar solution performs better under Simple Faecal Flotation Technique compared to Modified Centrifugal Faecal Flotation Technique suggesting that the solution is efficient in the diagnosis of this parasite elements using Simple Flotation technique.

Simple flotation technique using salt/sugar is highly sensitive compared to the sensitivity given by Modified Centrifugal Faecal Flotation technique.

For MCFF technique, the use of sodium chloride solution and brine is not efficient judging by their poor performance in this study. The performance of zinc sulphate solution under this technique was far below expectation as previous reports indicated that this solution recovered more parasite eggs [5]. This could be because zinc sulphate floated more debris which obstructed the view of parasite elements under the microscope and for sodium chloride solution and brine poor clarity of these solutions contributed to the low sensitivity.

Strongyle eggs, *Neoascaris vitulorum* and *Eimeria* oocysts were best diagnosed using salt solution and salt/sugar under Mini-FLOTAC technique compared to the Modified Centrifugal Faecal Flotation technique. This finding shows that Mini-FLOTAC is highly sensitive and efficient for these parasite elements.

The higher number of *Strongyloides* eggs detected using zinc sulphate solution with Mini-FLOTAC shows this solution is efficient for the parasite element compared to Modified Centrifugal Faecal Flotation technique (MCFF). Also, the performance of brine solution under the Mini-FLOTAC technique in the detection of *Moniezia benedeni* eggs indicated that in the diagnosis of this parasite element, the use of flotation solution with higher specific gravity is not needed.

Findings from this study clearly indicated that Mini-FLOTAC technique using sugar as floatation solution performs better in the diagnosis of *Capillaria* eggs, compared to the Modified Centrifugal Faecal Flotation using the same

flotation solution.

The sensitivity of Mini-FLOTAC using salt/sugar solution is higher compared to the salt/sugar Modified Centrifugal Faecal Flotation. This diagnostic assessment clearly indicated that the solution was the best under the Mini-FLOTAC technique.

The most time-consuming technique for sample processing was Modified Centrifugal Faecal Flotation while Mini-FLOTAC was the quickest to process followed by simple flotation technique. The waiting time for the clarification of the parasite elements for simple flotation and centrifugation for Modified Centrifugal Faecal Flotation were factors accounted mostly for the length of processing single samples. Reading time was faster for salt/sugar, sugar, salt and brine solutions under the three techniques because of their clarity under the microscope with salt/sugar been the best.

The clarity of zinc sulphate solution was distorted under the Modified Centrifugal Faecal Flotation technique which affect the reading time (i.e. time for parasite elements examination under the microscope). Factors such as production fault and the large number of faecal debris could be responsible since cattle sampled feed majorly on grass without concentrate supplementation.

In general, diagnostic assessment of the five flotation solutions clearly indicated that salt/sugar solution gave the optimum results under each faecal test for the three techniques compared to other solutions suggesting that the solution is highly efficient in the diagnosis of parasite elements, salt/sugar is easy to prepare, the solute is readily available, does not grow mouldy like sugar solution, does not crystalize or form cast, does not require preservative, it is not sticky or messy to work with, non-toxic to the environment and finally it has excellent clarity under the microscope.

One plausible explanation for the superior performance of the salt/sugar solution under each technique is that salt helps in homogenization of faeces and sugar which is more viscous make it clearer. This results in clearer slides with increasing chance of parasite elements detection [24].

For the diagnostic techniques, Mini-FLOTAC produced optimum results compared to the other two conventional techniques (Simple Faecal Flotation and Modified Centrifugal Faecal Flotation) especially with the use of salt/sugar as flotation solution. Also, Mini-FLOTAC apparatus is heat resistant, re-useable after thorough washing, has excellent clarity under the microscope, does not require centrifugation and is good for both qualitative and quantitative faecal analysis.

Findings from this study showed that only salt/sugar solution was highly efficient in detecting the eleven genera of bovine gastrointestinal parasites identified in this study using Mini-FLOTAC technique. This further support RVC/FAO recommendation that salt/sugar flotation solution is a general-purpose flotation solution.

This is the first-time salt/sugar and sugar flotation solutions were used with Mini-FLOTAC technique, just recently salt/glucose was used by the inventors [10] on a test running scheme, salt solution (NaCl) and zinc sulphate are the only two flotation solutions used for the Mini-FLOTAC technique [25].

The excellent performance of salt/sugar solution under Mini-FLOTAC technique from this study support the report by [25] that the sensitivity of the Mini-FLOTAC technique is highly dependent on the flotation solution used.

In terms of diagnosis of co-infection, Mini-FLOTAC technique performed better than the other two techniques showing that Mini-FLOTAC is a more sensitive technique. The variation in the number of parasite elements detected across the three techniques using sodium chloride and brine solution at specific gravity of (1.20), zinc sulphate and salt/sugar at specific gravity of (1.30) also support the finding of Cringoli et al. [8] that different flotation solutions with the same specific gravity do not produce the same results with respect to the same parasite elements, even when the same technique is used.

The only protozoa parasite detected by the three parasitological techniques is *Eimeria* oocyst which shows that it is the most prevalence protozoa parasite in the sampled area. This finding is similar to that of [10] which reported *Eimeria* oocyst as the only protozoan parasite detected using Mini-FLOTAC technique. There is no doubt that the gains in sensitivity provided by this technique should improve the laboratory diagnosis and control of bovine parasitic infections.

In conclusion, routine screening is a must for livestock production. The use of Mini-FLOTAC needs to be promoted in developing countries particularly in the laboratory with resource-limited settings. Based on the performance of salt/sugar flotation solution under each technique for this study, the use of salt/sugar as flotation solution in

the laboratory is recommended. For routine diagnosis, Mini-FLOTAC can be adopted because of the ease of usage.

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